

CD4⁺ T Cell Kinetics and Activation in Human Immunodeficiency Virus–Infected Patients Who Remain Viremic Despite Long-Term Treatment with Protease Inhibitor–Based Therapy

Steven G. Deeks,¹ Rebecca Hoh,¹ Robert M. Grant,² Terri Wrin,⁴ Jason D. Barbour,² Amy Narvaez,¹ Denise Cesar,⁵ Ken Abe,⁵ Mary Beth Hanley,² Nicholas S. Hellmann,⁴ Christos J. Petropoulos,⁴ Joseph M. McCune,^{1,2,3} and Marc K. Hellerstein^{1,5}

¹Department of Medicine, University of California at San Francisco and San Francisco General Hospital, ²Gladstone Institute of Virology and Immunology, and ³Department of Microbiology and Immunology, University of California at San Francisco; ⁴ViroLogic, South San Francisco; and ⁵Department of Nutritional Sciences, University of California at Berkeley

T cell dynamics were studied in human immunodeficiency virus–infected patients who continued using antiretroviral therapy despite detectable plasma viremia (RNA copies >2500 /mL). CD4⁺ cell fractional replacement rates, measured by the deuterated glucose technique, were lower in treated patients with detectable viremia than in untreated patients and were similar to those in patients with undetectable viremia. Cell cycle and activation markers exhibited similar trends. For any level of viremia, CD4⁺ cell fractional replacement rates were lower in patients with drug-resistant virus than in patients with wild-type virus, which suggests that the resistant variant was less virulent. Interruption of treatment in patients with drug-resistant viremia resulted in increased CD4⁺ cell activation, increased CD4⁺ cell turnover, and decreased CD4⁺ cell counts. These data indicate that partial virus suppression reduces CD4⁺ cell turnover and activation, thereby resulting in sustained CD4⁺ cell gains, and that measurements of T cell dynamics may provide an in vivo marker of viral virulence.

Combination antiretroviral therapy results in sustained increases in CD4⁺ T cell counts and slowing of disease progression [1–3]. Although these immunologic and clinical benefits are related to suppression of human immunodeficiency virus (HIV) replication, the degree to which suppression of plasma viremia to undetectable levels is required for durable immunologic benefit is not known [4–7]. Indeed, in patients continuing a protease inhibitor–based regimen despite detectable viremia, CD4⁺ T cell counts often remain elevated above the pretherapy nadirs. CD4⁺ T cell changes in these patients are associated with the magni-

tude of reduction in plasma HIV-1 RNA levels below the pretherapy steady-state level of viremia and not by the absolute level of viremia achieved [4]. These observations indicate that partial suppression of viral replication in some manner alters the long-term dynamics of CD4⁺ T cell production, destruction, or distribution and thereby results in a durable increase in CD4⁺ T cells.

This apparent “disconnect” between incomplete suppression of HIV-1 replication (“virologic failure”) on the one hand and immunologic benefit on the other has generated uncertainty regarding the clinical management of such patients. Measurement of T cell kinetics and T cell activation might be useful in this regard. Using deuterated glucose to label DNA in actively proliferating CD4⁺ T cells, we showed earlier that untreated HIV infection is associated with accelerated CD4⁺ T cell replacement (i.e., shorter T cell survival time) and limited or no compensatory increase in absolute production rate of new cells [8–10]. Durable suppression of plasma viremia to undetectable levels for 12–24 months results in longer CD4⁺ T cell survival and restoration of near-normal CD4⁺ T cell kinetics [9]. T cell activation shows a similar pattern, with elevation of activation during untreated HIV-1 infection and lowering of activation by durable virus suppression [11–15]. The effects on T cell kinetics and activation of continuing combination therapy, despite detectable viremia (virologic failure), have not been determined.

Interruption of therapy has proven to be a useful strategy in studying the pathogenesis of treated and untreated HIV disease. We recently investigated the virologic consequences of prolonged incomplete virus suppression (plasma HIV RNA level >2500 copies/mL) by interrupting therapy in a controlled man-

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This study was approved by the institutional review board of the University of California at San Francisco (USCF). All patients gave written informed consent.

The phenotypic drug susceptibility and replication capacity assays were performed at ViroLogic.

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Reprints or correspondence: Dr. Steven G. Deeks, San Francisco General Hospital, 995 Potrero Ave., San Francisco, CA 94110 (sdeeks@php.ucsf.edu).

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ner [7]. Recombinant viruses containing patient-derived reverse transcriptase and protease sequences demonstrated significant reductions in replicative capacity in the absence of drug, whereas discontinuation of therapy for 12 weeks resulted in the emergence of wild-type HIV-1 with increased replicative capacity. This was temporally associated with increased levels of plasma HIV-1 RNA and decreased peripheral CD4⁺ T cell counts. Whether reduced viral replicative capacity in vitro has any functional consequences on T cell survival, production, or activation in vivo was not addressed in our initial report.

Here, we sought to establish the immunologic basis for sustained CD4⁺ T cell increases in patients continuing to receive therapy despite significant levels of plasma viremia (plasma HIV RNA levels >2500 copies/mL). Two studies were done to address this question. The first study was a cross-sectional analysis of 3 groups of HIV-1-infected patients. The second study examined the effects of a 12-week treatment interruption in a group of patients with detectable viremia despite prolonged treatment with protease inhibitor therapy. In each study, deuterated glucose was used as a marker of CD4⁺ T cell production, to measure CD4⁺ T cell kinetics by labeling and quantifying replicating DNA [8, 9, 16]. CD4⁺ T cell activation (CD38⁺DR⁺) and cell cycle markers (Ki67) were also studied.

Methods

Study design: cross-sectional study. The first part of this study was cross-sectional. Eligibility criteria included a documented CD4⁺ T cell nadir of <350 cells/ μ L and availability of \geq 18 months of prior treatment history. Three treatment groups were studied: (1) the virologic failure group, which had received continuous protease inhibitor-based therapy during the preceding 18 months and had >2500 HIV-1 RNA copies/mL during the preceding 24 weeks; (2) the virologic success group, which had received continuous protease inhibitor-based therapy during the preceding 18 months and had <50 HIV-1 RNA copies/mL during the preceding 24 weeks; and (3) the no treatment group, which had received no antiretroviral therapy during the preceding 12 months and had >2500 HIV-1 RNA copies/mL at study entry. Exclusion criteria included concurrent or prior use of immunomodulating therapy (including hydroxyurea and/or systemic chemotherapy) or evidence of a serious infection within the preceding 3 months. All patients underwent a single evaluation.

Patients for this cross-sectional study were recruited from patients followed up at San Francisco General Hospital. In addition, data obtained at the time patients enrolled in our treatment-interruption study [7] were used in this cross-sectional analysis. These patients all met the eligibility for the virologic failure group described above.

A subset of patients experiencing prolonged virologic failure of a protease inhibitor-containing regimen underwent evaluation, using the deuterated glucose labeling technique (see below). We compared data generated from these patients with data for a cohort of patients, described elsewhere, who were untreated or who had achieved long-term virus suppression with protease inhibitor therapy [9].

Study design: treatment interruption. We also performed a prospective study evaluating the consequences of discontinuing therapy after long-term incomplete virus suppression [7]. Eligibility criteria included continuous protease inhibitor therapy for \geq 12 months preceding study entry, stable antiretroviral regimen during the preceding 4 months, and a documented HIV-1 RNA virus load of >2500 copies/mL for the preceding 6 months. All patients underwent 2 baseline evaluations. At day 0, all antiretroviral therapy was discontinued. Patients were then seen weekly for 12 weeks, as described elsewhere [7].

Virus measurements. HIV-1 RNA levels were determined, using EDTA-anticoagulated blood plasma assayed in batch, by use of a branched DNA assay (Quantiplex HIV-1 bDNA, version 3.0; Chiron) with a lower limit of quantification of 50 copies RNA/mL. All assays were done at a central laboratory (Bayer Diagnostics, Emeryville, CA).

Phenotypic susceptibility to antiretroviral therapy was determined by using a recombinant virus-based assay (Phenosense; ViroLogic), as described elsewhere [17]. Viral replication capacity was measured with a modified version of this phenotypic resistance assay, in which the degree of replication of the test and control vectors was measured in the absence of drug, and the readout was normalized for viral inoculum. Results are expressed as a ratio between the test and control vectors, such that a value <1 reflects reduced replicative capacity [7].

T cell phenotypic studies. Three-color flow cytometry was done by use of freshly collected heparin-anticoagulated whole blood. T cells were identified by CD3 expression. Activated T cells were identified by the activation markers CD38 and HLA-DR. Naive T cells were defined as CD45RA⁺CD62L⁺, whereas memory/effector cells were defined as those that did not express CD45RA or CD62L [9]. Ki67 expression was assessed by flow cytometry, using rabbit anti-human Ki67 (Dako), as described elsewhere [18].

Measurement of T cell kinetics in vivo with the deuterated glucose labeling technique. The proliferation and replacement rates of CD4⁺ T cells were measured by the [²H]glucose/fluorescence-activated cell sorter (FACS; Becton Dickinson)/mass spectrometric method (Agilent), as described in detail elsewhere [8, 9]. In brief, subjects received 160 g of [6,6-²H₂]glucose, infused over 24 h. Blood samples were obtained during the infusion (every 6 h) for blood glucose enrichments and then between days 4–6 and 7–10 for T cell isolation. CD4⁺ T cells, both total and the memory/effector phenotype subpopulation, were sort-purified by multiparameter FACS. Genomic DNA was isolated from the pelleted cells, hydrolyzed to free nucleosides, and derivatized for gas chromatographic/mass spectrometric analysis. The isotopic enrichment of [²H₂]deoxyadenosine was determined by comparison to labeled standards [9]. The fractional replacement rate constant (*k*) of each cell population was calculated on the basis of precursor-product relationship, using corrected blood glucose enrichments to represent the precursor pool [8, 9, 16]. Absolute production rates of circulating T cells (cells/ μ L per day) were calculated by multiplying *k* (*d*⁻¹) by the circulating pool size (cells/ μ L). The mean half-life of CD4⁺ T cells was calculated as 0.693/*k* [9].

To infer T cell death or removal rates directly from label incorporation data, a steady-state pool size must be present in the peripheral circulation (i.e., if the circulating pool size is constant, every

newly divided T cell in the peripheral blood is balanced by the loss of a previously existing cell). In patients whose antiretroviral therapy was discontinued, CD4⁺ T cell counts continued to decline throughout the 12-week treatment-interruption period, including the period of the isotope-labeling study. Accordingly, we applied a non-steady-state correction (modified Steele equation) [19] to calculate the CD4⁺ T cell fractional removal rate from the blood (k_d). CD4⁺ T cell counts were monitored every week during the period 4–6 weeks before to 2 weeks after the infusion, and the interval change in the CD4⁺ T cell counts was measured for each subject. k_d was calculated from the measured production rate (based on label incorporation) and the rate of change in the circulating pool size (i.e., removal rate [cells/ μ L per day] = production rate [cells/ μ L per day] – change in pool size [cells/ μ L per day]): k_d (d^{-1}) = [production rate (cells/ μ L per day) – change in pool size (cells/ μ L per day)]/mean interval pool size (cells/ μ L), where a decrease in pool size is expressed as a negative number.

We have observed no differences in T cell labeling kinetics between 24- and 48-h intravenous [²H]glucose infusions (unpublished observations). The proportion of labeled T cells after pulse [²H]glucose administration generally remains stable for the first 7–10 days [8, 9], so we analyzed 2 time points during that period.

Statistical analysis. Median values (with interquartile ranges [IQRs]) were determined for all variables. Nonparametric methods were used for univariate, comparison, and correlation tests. Differences in variables between patient groups were analyzed with the Mann-Whitney *U* test. All correlations were determined by use of Spearman’s rank correlation. All *P* values were 2-sided. Statistical analyses were done by use of SPSS software (version 8.0; SPSS).

Results

Cross-sectional study of 3 treatment groups. In total, 71 patients were included in our cross-sectional study: 36 were in the virologic failure group and 18 and 17 in the virologic success and untreated groups, respectively (table 1). The pretreatment CD4⁺ T cell nadir was similar among the 3 groups: The virologic failure and success groups had median pretherapy CD4⁺ T cell counts of 142 and 215 cells/mm³, respectively, and the untreated patients had 162 cells/mm³. The median change in CD4⁺ T cell count between initiation of protease inhibitor therapy and study entry was 277 cells/mm³ in the virologic success group and 109 cells/mm³ in the virologic failure group. Patients in the virologic failure group had detectable viremia while receiving a protease inhibitor-containing regimen for a median of 28 continuous months preceding study entry (table 1).

Virus obtained from plasma of patients experiencing virologic failure exhibited moderate to high reductions in phenotypic drug susceptibility, as we have reported elsewhere [7]. The median decrease at study entry in susceptibility to the protease inhibitor used was 24-fold (IQR, 12–58-fold). The median viral replicative capacity was 0.12 (IQR, 0.09–0.18) for the virologic failure group and 0.62 (IQR, 0.49–0.68) for untreated patients (*P* < .001).

CD4⁺ T cell phenotype and cell cycle markers in the 3 treatment groups. The level of CD4⁺ T cell activation differed significantly across the 3 treatment groups (figure 1). The median percentages of CD4⁺ T cells coexpressing CD38 and HLA-DR

Table 1. Characteristics of 3 groups of human immunodeficiency virus (HIV)–1–infected adults receiving 3 different treatment regimens.

Characteristic	Treated		
	Untreated (<i>n</i> = 17)	Virologic failure (<i>n</i> = 36)	Virologic success (<i>n</i> = 18)
Age, years	41.5	40.7	42.9
Pre–protease inhibitor CD4 ⁺ T cell count, cells/mm ³	NA	142 (87–202)	215 (105–287)
Pre–protease inhibitor plasma HIV RNA, log ₁₀ copies/mL	NA	4.81 (4.50–5.11)	4.98 (4.36–5.15)
Duration of protease inhibitor therapy, months	NA	32.1 (28.7–6.5)	32.9 (29.0–38.9)
Time elapsed since virologic failure, months	NA	27.8 (24.6–32.1)	NA
Change in CD4 ⁺ T cell count/mm ³ , pretherapy to study entry	NA	+109 (56–197)	+277 (143–356)
CD4 ⁺ T cell count/mm ³ at study entry	162 (128–244)	258 (205–316)	478 (342–597)
CD8 ⁺ T cell count/mm ³ at study entry	721 (620–952)	989 (613–1402)	767 (637–928)
Plasma HIV RNA levels at study entry, log ₁₀ copies RNA/mL	5.33 (4.71–5.38)	4.35 (3.83–4.63)	1.7 (<1.7)
% Naive CD4 ⁺ T cells	16.5 (13–25.5)	22.1 (14.9–33)	23.2 (14.4–38.8)
Naive CD4 ⁺ T cell count/mm ³	34 (23–52)	56 (30–96)	101 (56–251)
% Memory/effector CD4 ⁺ T cells	83.5 (74.6–87.1)	78 (67–85.1)	76.9 (61.3–85.6)
Memory/effector CD4 ⁺ T cell count/mm ³	140 (92–205)	170 (141–256)	315 (222–384)
% Naive CD8 ⁺ T cells	11.7 (4–16)	8.8 (6–16.6)	11.2 (4.3–22.9)
Naive CD8 ⁺ T cell count/mm ³	59 (38–108)	103 (39–59)	83 (23–159)
% Memory/effector CD8 ⁺ T cells	88.3 (84–96.1)	91.3 (83.5–94)	88.7 (77.2–95.7)
Memory/effector CD8 ⁺ T cell count/mm ³	640 (455–739)	833 (610–1246)	644 (517–866)

NOTE. Values are median (25th–75th interquartile range). Characteristics of patients in the 3 study groups at the time of the study were as follows: virologic failure group, plasma HIV RNA level >2500 copies/mL while receiving stable therapy with a protease inhibitor–based regimen; virologic success group, plasma HIV RNA level <50 copies/mL while receiving stable therapy with a protease inhibitor–based regimen; and untreated group, plasma HIV RNA level >2500 copies/mL and no antiretroviral therapy. Pretherapy, date protease inhibitor therapy was initiated (these data were obtained retrospectively through chart review); virologic failure, time of documentation of viremia (HIV RNA level >2500 copies/mL). Naive cell populations were defined as those coexpressing CD45RA and 62L, and memory/effector cell populations were defined as those with the following phenotypes: CD45RA⁺62L[–], CD45RA[–]62L⁺, and CD45RA[–]62L[–]. NA, not applicable.

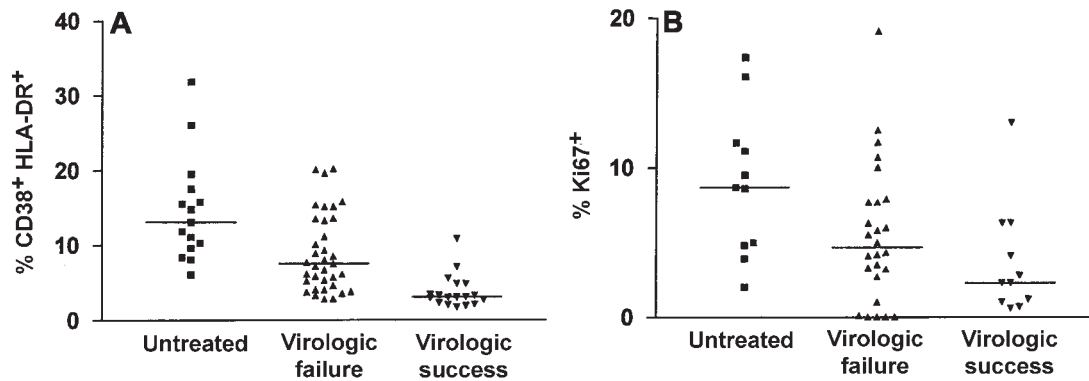


Figure 1. Expression of cell activation (CD38⁺HLA-DR⁺) and cell cycle (Ki67⁺) markers in peripheral T cells from human immunodeficiency virus (HIV)-infected patients with various antiretroviral treatment responses. Data for 3 treatment groups are shown. *A*, Percentage of CD4⁺ T cells coexpressing CD38 and HLA-DR in untreated ($n = 15$), virologic failure (plasma HIV RNA levels >2500 copies/mL during long-term treatment; $n = 36$), and virologic success (plasma HIV RNA levels <50 copies/mL during long-term treatment; $n = 18$) groups. In each pairwise comparison, the differences between groups were significant ($P < .01$ for each; Wilcoxon rank sum test). *B*, Percentage of CD4⁺ T cells expressing Ki67 for the untreated ($n = 11$), virologic failure ($n = 27$), and virologic success ($n = 11$) groups. In pairwise comparisons, the difference between the untreated patients and either of the 2 treated groups was significant ($P = .04$ and $P = .01$, respectively; Wilcoxon rank sum test). There was no significant difference between the patients experiencing virologic failure vs. virologic success ($P = .4$).

were 13.1%, 7.6%, and 3.1% (untreated, virologic failure, and virologic success groups, respectively; $P < .01$ for all pairwise comparisons; figure 1).

The median percentage of CD4⁺ T cells expressing Ki67 antigen also varied across the 3 groups (figure 1*B*), with values highest among the untreated patients (8.7%), intermediate among patients with virologic failure (4.3%), and lowest in the virologic success group (2.3%). The difference between the virologic failure and success groups was not significant ($P = .4$). The percentage of CD4⁺ T cells expressing Ki67 was significantly higher in the untreated patients than in patients in the 2 treated groups ($P = .04$ for untreated group vs. virologic failure group; $P = .01$ for untreated group vs. virologic success group).

The percentage of CD4⁺ T cells expressing either naive or memory/effector cell surface markers was similar across the 3 treatment groups (table 1). However, the absolute number of naive CD4⁺ T cells differed among the 3 groups and was highest in the virologic success group, intermediate in the virologic failure group, and lowest in the untreated group ($P = .02$ for virologic failure group vs. success group; $P = .06$ for virologic failure group vs. untreated group; $P = .002$ for virologic success group vs. untreated group). Similar trends were observed with the absolute number of memory/effector CD4⁺ T cells ($P < .10$ for each pairwise comparison).

CD8⁺ T cell activation markers and cell cycle markers in the 3 treatment groups. The level of CD8⁺ T cell activation was assessed in the 3 treatment groups. The median percentages of CD8⁺ T cells coexpressing CD38 and HLA-DR were 24.2%, 13.9%, and 4.2% (untreated, virologic failure, and virologic success groups, respectively; $P < .01$ for each pairwise comparison). The median percentage of CD8⁺ T cells expressing Ki67 antigen

also varied across the 3 groups, with values being highest among the untreated patients (4.4%), intermediate among patients with virologic failure (3.5%), and lowest in the virologic success group (2.5%). The differences among these 3 groups were not statistically significant ($P > .1$ for each pairwise comparison).

CD4⁺ T cell kinetics in treated patients with detectable plasma viremia. Using the [²H]glucose/FACS/mass spectrometric technique to measure cell proliferation [8, 9], we analyzed CD4⁺ T cell turnover in a group of 17 patients experiencing virologic failure while receiving a protease inhibitor-based treatment regimen (median CD4⁺ T cell count, 295 cells/mm³ [IQR, 236–358 cells/mm³]; median HIV-1 RNA level, 4.37 log₁₀ copies/mL [IQR, 3.89–4.93 log₁₀ copies/mL]). CD4⁺ T cell counts in this group were 138 cells/mm³ above their pretherapy levels (IQR, 77–233 cells/mm³). The median k of the total peripheral CD4⁺ T cell population was 0.010 d⁻¹ (IQR, 0.008–0.014 d⁻¹; table 2).

Figure 2 compares values from individual subjects with previously reported [9] k from patients who were untreated ($n = 10$; median CD4⁺ T cell count, 243 cells/mm³; plasma HIV-1 RNA level, 4.63 log₁₀ copies/mL; mean CD4⁺ T cell k , 0.032 d⁻¹) or who had achieved long-term virus suppression on a protease inhibitor-based regimen ($n = 8$; median CD4⁺ T cell count, 487 cells/mm³; CD4⁺ T cell k , 0.009 d⁻¹). The k of CD4⁺ T cells was significantly lower in the virologic failure group than in the untreated group ($P < .001$); however, the difference between the virologic failure and virologic success groups was not significant ($P = .32$). The median CD4⁺ T cell half-lives for the 3 groups were 68 days (virologic failure), 22 days (untreated), and 82 days (virologic success).

Similar results were observed for the kinetics of memory/effector CD4⁺ T cells, with significantly higher k rates in the

Table 2. T cell turnover in 3 groups of human immunodeficiency virus (HIV)-1-infected adults receiving different treatment regimens.

Parameter	Untreated	Treated	
		Virologic failure	Virologic success
CD4 ⁺ T cell fractional replacement rate, (<i>k</i>) (<i>d</i> ⁻¹)	0.032 (0.023–0.036) (<i>n</i> = 10)	0.010 (0.008–0.014) (<i>n</i> = 17)	0.009 (0.007–0.011) (<i>n</i> = 8)
Absolute CD4 ⁺ T cell production, cells/ μ L/day	6.6 (5.7–8.8) (<i>n</i> = 10)	2.8 (1.9–3.3) (<i>n</i> = 17)	3.6 (2.9–5.3) (<i>n</i> = 8)
Memory/effector CD4 ⁺ T cell fractional replacement rate, (<i>k</i>) (<i>d</i> ⁻¹)	0.026 (0.021–0.031) (<i>n</i> = 8)	0.012 (0.006–0.016) (<i>n</i> = 10)	0.011 (0.009–0.014) (<i>n</i> = 8)
CD8 ⁺ T cell fractional replacement rate, (<i>k</i>) (<i>d</i> ⁻¹)	0.028 (0.022–0.033) (<i>n</i> = 8)	0.007 (0.005–0.01) (<i>n</i> = 16)	0.005 (0.004–0.014) (<i>n</i> = 7)
Absolute CD8 ⁺ T cell production, cells/ μ L/day	23 (11.4–30.1) (<i>n</i> = 8)	7 (4.7–13.6) (<i>n</i> = 15)	5.6 (3.4–9.8) (<i>n</i> = 7)
Memory/effector CD8 ⁺ T cell fractional replacement rate, (<i>k</i>) (<i>d</i> ⁻¹)	0.031 (0.022–0.032) (<i>n</i> = 12)	0.011 (0.009–0.013) (<i>n</i> = 15)	0.01 (0.005–0.014) (<i>n</i> = 6)

NOTE. Values are median (interquartile range). CD4⁺ and CD8⁺ T cell kinetics are shown for treated patients experiencing virologic failure (plasma HIV RNA level >2500 copies/mL during long-term protease inhibitor treatment) and are compared with data reported earlier for untreated patients or treated patients experiencing long-term virus suppression (plasma HIV RNA level <50 copies/mL) [9]. For each parameter tested, the difference between the virologic failure group and the untreated group was significant (*P* ≤ .01), whereas the difference between the virologic failure and virologic success groups was not (*P* > .10).

untreated group than in either treated group but no significant difference between the virologic failure and success groups (table 2).

CD8⁺ T cell kinetics in treated patients with detectable plasma viremia. Kinetic results for CD8⁺ T cells and memory/effector CD8⁺ T cells paralleled the results for CD4⁺ T cells (table 2). The *k* of CD8⁺ T cells and of memory/effector CD8⁺ T cells was significantly lower in the virologic failure group than in the untreated group (*P* < .01), whereas the difference between the virologic failure and virologic success groups was not significant.

CD4⁺ T cell kinetics in patients with drug-resistant versus wild-type viremia. The relationship between the level of viremia (log₁₀ HIV RNA copies/mL) and CD4⁺ T cell *k* was evaluated (figure 2*B*). Plasma virus from all patients in the virologic failure group had phenotypic evidence of protease inhibitor resistance. For any given level of plasma viremia, *k* was lower in treated patients with drug-resistant viremia than in untreated patients. This difference was particularly evident when plasma HIV RNA levels were >4.5 log₁₀ RNA copies/mL of plasma.

The effects of treatment interruption. Eighteen patients underwent a prospective 12-week treatment interruption. As detailed

elsewhere [7], treatment interruption for 12 weeks was associated with decreased CD4⁺ T cell counts (median decrease, 89 cells/mm³), increased plasma HIV-1 RNA levels (median increase, 0.87 log₁₀ copies/mL), and the emergence of HIV-1 with increased replicative capacity (median, from 0.26 to 0.63; *P* = .001). Although the percentage of CD4⁺ T cells expressing either a naive or a memory/effector phenotype did not change during treatment interruption (median, from 20.2% to 16.5% for naive CD4 T cells and from 79.8% to 83.5% for memory CD4 T cells), the absolute number of both naive and memory/effector phenotype CD4⁺ T cells decreased, with most of the reduction in total CD4⁺ T cells in the memory/effector population (median, from 36 to 31 cells/mm³ for change in absolute number of naive CD4⁺ T cells [*P* = .004] and from 162 to 100 cells/mm³ for change in absolute number of memory/effector CD4⁺ T cells [*P* = .001]). Counts of total and naive phenotype (percentage or absolute) CD8⁺ T cells and memory/effector phenotype CD8⁺ T cells (percentage or absolute) remained stable during the 12-week treatment interruption (data not shown).

During the 12-week treatment interruption, the percentage of CD4⁺ T cells coexpressing CD38 and HLA-DR increased sig-

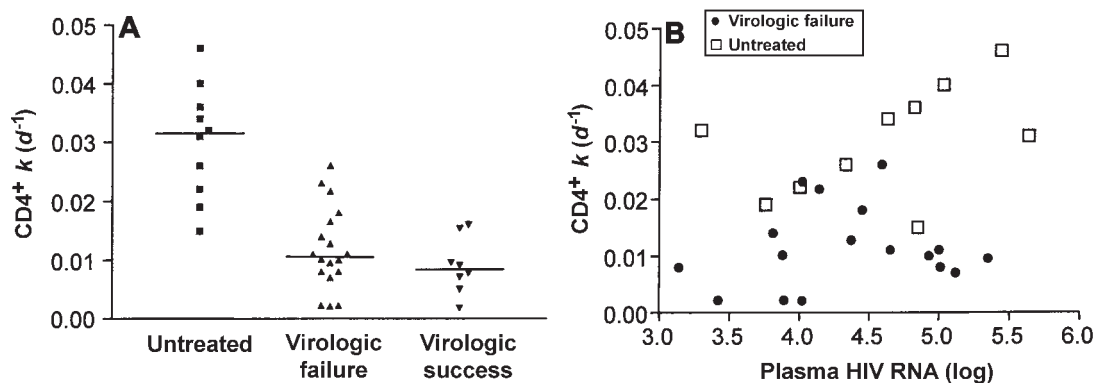


Figure 2. A, Fractional replacement rates (*k*) of CD4⁺ T cells are shown for each of 3 treatment groups of human immunodeficiency virus (HIV)-infected patients (untreated, virologic failure, and virologic success). Data for the untreated and virologic success groups have been reported elsewhere [9]. B, *k* vs. the absolute level of viremia (log₁₀ copies/mL) for the 2 treatment groups with detectable viremia (virologic failure and untreated).

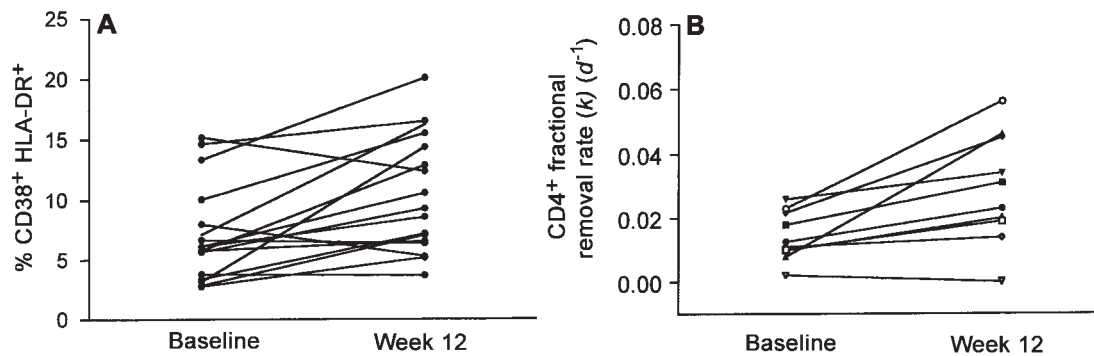


Figure 3. Effect of a 12-week treatment interruption on CD4⁺ T cell activation markers (A; $n = 18$) and on CD4⁺ T cell fractional removal rate (B; $n = 9$) in human immunodeficiency virus (HIV)–infected patients. At the time therapy was discontinued, patients were in a state of long-term virologic failure (HIV RNA level >2500 copies/mL) while receiving a protease inhibitor–based regimen. The differences between baseline and week 12 were significant ($P = .006$ for percentage of CD4⁺ T cells coexpressing CD38 and HLA-DR and $P = .01$ for fractional removal rate).

nificantly (median, 6.1% to 9.3%; $P = .006$; figure 3A). There was a nonsignificant increase in the percentage of CD8 T cells coexpressing CD38 and HLA-DR (median, 13% to 20.1%; $P = .13$).

Nine subjects agreed at the time of enrollment to have repeated measurements of CD4⁺ T cell kinetics after the 12-week treatment interruption. After correcting for the non–steady-state decline in CD4⁺ T cells during the infusion study period, the fractional removal rate (k_d) of peripheral CD4⁺ T cells after 12 weeks of treatment interruption was $0.023 \pm 0.017 d^{-1}$ (IQR, 0.019–0.034 d^{-1}), which was significantly higher than the value obtained before treatment interruption ($0.011 d^{-1}$ [IQR, 0.01–0.018 d^{-1}]; $P = .01$; figure 3B). There was no change in k_d for CD8⁺ T cells (data not shown).

Discussion

The immunologic consequences of partial virus suppression with a protease inhibitor–based regimen have not been fully established. Here, we measured host-related immunologic factors (including proliferation, removal, and activation of circulating T lymphocytes) and their relationship to the virologic response to therapy or to its discontinuation. A number of interesting findings emerged. First, the k values of CD4⁺ T cells in long-term treated patients with detectable plasma viremia (plasma HIV RNA levels >2500 copies/mL) were lower than those we observed earlier in untreated patients and were similar to values found in long-term treated patients with undetectable levels of plasma viremia [9]. Second, CD4⁺ T cell activation and cell cycle markers also were lower in treated patients with detectable plasma viremia than in untreated patients and were similar to levels observed in treated patients with undetectable levels of plasma viremia. Third, for any given level of viremia, the CD4⁺ T cell k —a possible *in vivo* marker of virulence—was lower in treated patients with drug-resistant viremia than in untreated patients with wild-type viremia. Last, when treatment was interrupted for 12 weeks after a

period of prolonged virologic failure, HIV-1 RNA levels increased, and this was associated with increased CD4⁺ T cell activation and an accelerated removal rate of circulating CD4⁺ T cells.

Collectively, these observations suggest that partial virus suppression with a protease inhibitor–based regimen results in a state of relative immunologic quiescence, in which T cell dynamics are similar but not identical to those observed in successfully treated patients. Thus, the apparent benefits of effective antiretroviral therapy for CD4⁺ T cell dynamics can be achieved despite the continued presence of detectable plasma viremia, at least during 2–3 years of follow-up.

The interpretation of CD4⁺ T cell kinetics in the context of both untreated and treated HIV infection remains controversial [10]. In our current analysis of patients with prolonged virologic failure, we assumed that patients had achieved steady state in CD4⁺ T cell pools. Thus, at the time of deuterated glucose labeling, the number of peripheral CD4⁺ T cells being produced was equal to the number of CD4⁺ T cells being removed, and the fraction of CD4⁺ T cells incorporating deuterated glucose into DNA was balanced by an equal fraction of CD4⁺ T cells being destroyed. Compared with the untreated patients studied earlier [9], we observed that the relative fraction of cells actively being produced and removed in the virologic failure group was low. Indeed, this low turnover state was similar to that observed earlier by our group in patients achieving long-term virus suppression (HIV RNA levels <50 copies/mL) while receiving combination therapy [9].

The sustained CD4⁺ T cell increases during treatment with a partially suppressive regimen might be attributed to increased CD4⁺ T cell production, decreased CD4⁺ T cell destruction (i.e., prolonged survival), or both. Our labeling data indicating that CD4⁺ T cells exhibit slower turnover in patients with partial virus suppression suggest prolonged T cell survival as the primary mechanism underlying the sustained CD4 cell increase

often observed in these patients. The fraction of CD4⁺ T cells expressing markers of activation (i.e., CD38⁺ HLA-DR⁺) and the fraction of cells actively cycling (i.e., Ki67⁺) demonstrated similar trends, with these fractions being much lower during partial virus suppression than in untreated patients. Last, when compared with untreated patients, patients experiencing a partial virologic response to therapy had a CD4⁺ T cell population that was enriched for naive CD4⁺ T cells, which are known to have a prolonged survival time [9]. Each of these observations—low turnover, less activation, and greater numbers of naive cells—is consistent with a greater mean life span for peripheral CD4⁺ T cells.

Longer survival of peripheral CD4⁺ T cells may, in turn, have several explanations. First, reduced levels of viral replication and/or the emergence of a less virulent virus may lead to reduced CD4⁺ and CD8⁺ T cell activation and thus less activation-induced cell death [12, 20–23]. Second, chronic, diffuse T cell activation may suppress the normal homeostatic production of long-lived T cells by altering their activation threshold for cell division [22, 24]. Third, the emergence of a drug-resistant virus population that is less able to productively infect thymic tissue [25, 26] may allow for a greater production of long-lived naive and true memory CD4 cells. The first 2 mechanisms are supported by our observations that CD4 T cell half-life was longer in those patients with lower measures of T cell activation (i.e., virologic failure subjects vs. untreated subjects and pretreatment interruption subjects vs. posttreatment interruption subjects). The final mechanism is consistent with our recent observation that protease inhibitor-resistant viruses, compared with wild-type viruses, have attenuated replicative potential in human thymic tissue [25]. The observation that thymic output (as measured by the frequency of CD4⁺ T cells containing T cell receptor excision circles) predicts CD4⁺ T cell gains during virologic failure [27, 28], is also consistent with the latter hypothesized mechanism.

We currently cannot distinguish between these possible mechanisms, nor can we assess to what degree the immunologic benefit of partial virus suppression is due to altered pathogenicity of the drug-resistant virus or to the lower virus load present in each individual. Because plasma virus load and viral phenotype varied in parallel in both our cross-sectional and longitudinal studies, their independent causal roles cannot be distinguished. However, for any given level of viremia, T cell destruction rates were generally lower in treated patients with drug-resistant virus than in untreated patients with wild-type virus (figure 2B). This difference was particularly striking at plasma HIV RNA levels >4.5 log₁₀ copies/mL. These observations, although preliminary and limited by small sample size, suggest that drug-resistant virus has altered pathogenicity that is not fully explained by reduced *in vivo* replication.

At present, no laboratory markers are available to characterize the *in vivo* virulence, or immunopathogenicity, of an HIV-infected patient's predominant viral phenotype. *Ex vivo* replication capacity of a viral isolate does not necessarily reflect the

in vivo consequences for T lymphocyte survival or production. Our documentation of widely different CD4⁺ T cell kinetics (fraction replacement and destruction rates, reflecting longer average survival) in patients with drug-resistant versus wild-type virus isolates at similar levels of plasma viremia (figure 2B), therefore, represents a potential functional marker of virulence in patients who are viremic. If confirmed in other settings, this may prove to be a useful test for research studies or for clinical management of HIV-infected individuals with viremia.

Elevated levels of CD8⁺ T cell activation have been associated with accelerated disease progression in untreated patients [13]. The mechanism underlying this observation has not been elucidated. In particular, it is not clear whether a high CD8⁺ T cell activation level drives CD4⁺ T cell depletion and disease progression or whether high CD8⁺ T cell activation is an epiphenomenon associated with other factors driving disease progression. In the current study, we observed significantly higher levels of CD8⁺ T cell activation and turnover (*k*) in untreated patients with wild-type virus than in treated patients with resistant virus, which suggests that partial virus suppression and/or emergence of a less-fit virus results in less CD8⁺ T cell activation and death. When therapy was discontinued, CD8⁺ T cell activation levels increased, although the change was not significant. CD8⁺ T cell fractional destruction (*k_d*) and the total CD8⁺ T cell count did not change. We cannot offer a definitive explanation for the stability in CD8⁺ T cell counts and dynamics after treatment interruption, but we can offer some speculations. One possibility is that the initial effect of increased viremia with a wild-type virus acts directly on CD4⁺ T cells, whereas the effect on CD8⁺ T cells is indirect (i.e., requires emergence of increased antigenic load due to opportunistic infections). The sources of CD8⁺ T cells may also be different from those of CD4⁺ T cells and may be differentially affected by increased viremia with wild-type virus.

It might be argued that plasma HIV RNA levels less than or greater than 50 copies/mm³ represent an arbitrary distinction and that the virologic response to treatment should be considered to be a continuum. Of note, we observed differences between “complete” (i.e., plasma HIV RNA levels <50 copies/mL) and “incomplete” virologic suppression (i.e., plasma HIV RNA levels >2500 copies/mL). Patients achieving and maintaining undetectable levels of plasma viremia had greater treatment-mediated CD4⁺ T cell increases than those who had achieved only a partial virologic response. Furthermore, there were nonsignificant trends suggesting improved CD4⁺ T cell activation or dynamics (figures 1 and 2) in treated patients with undetectable plasma viremia compared with treated patients with detectable plasma viremia. Collectively, these observations are consistent with cohort data indicating that the greater the degree of virus suppression, the greater the degree of peripheral CD4⁺ T cell recovery [4, 29, 30]. Our observations, therefore, continue to support recommendations that durable suppression of plasma viremia to below the level of detection remains the standard goal of combination therapy.

The clinical implications of our findings remain to be established. Continuation of a drug regimen in the presence of ongoing viral replication will likely select for further drug resistance, thus limiting future options for more effective virus suppression. Presumably, unless more effective therapy is initiated, viral replication will increase over time and will be associated with increased CD4⁺ T cell activation, accelerated CD4⁺ T cell destruction, insufficient compensation by CD4⁺ T cell production, and decreasing peripheral CD4⁺ T cell counts. When or whether to switch therapy in treated patients with detectable viremia cannot be determined from our data. Nevertheless, these results are consistent with observational data that the natural history of HIV-1-mediated CD4⁺ T cell depletion is altered by the continued use of antiretroviral therapy, even when plasma viremia remains detectable [4, 6].

Last, the complexities inherent in labeling T cells *in vivo* should be recognized when interpreting our results. During any period of time, only a subpopulation of the T cell pool is actively dividing. The functional effects on CD4⁺ T cell pool size of any rate of cell division depends on the nature of the proliferation. Some T cells divide in response to an antigenic stimulus and are destined to die off rapidly, perhaps through activation-induced cell death [12–15, 21, 31]. Other cells may divide and differentiate into long-lived cells as part of “homeostatic” proliferation [22, 24, 31, 32]. If most cell proliferation in an individual is of the short-lived variety, the result will be a smaller pool size at steady state, compared with the same total proliferation rate in mainly long-lived cells. Thus, in our current study, a lower daily replacement fraction but larger pool size of CD4⁺ T cells in patients with virologic failure (vs. those with untreated HIV-1 infection) implies that the cells being produced have a longer life span. Direct testing of this kinetic explanation requires measurement of the die-away curves of labeled T cells in the 2 settings. Such measurements are now feasible [9] but were not performed in the present study.

In summary, our data indicate that failure of antiretroviral therapy to durably suppress plasma viremia to undetectable levels results in the emergence of a virus population with reduced replicative capacity *in vitro*, decreased levels of viral replication, and prolonged CD4⁺ T cell survival, compared with findings in untreated HIV-1 infection. These effects on T cell kinetics are similar but not identical to those observed with long-term complete virus suppression [9]. As a consequence, sustained increases in CD4⁺ T cell counts occur in patients with drug-resistant virus.

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